Taxonomic affinities and criteria for identification of the common ectendomycorrhizal symbiont of pines

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Evidence from cultured and soil-borne mycelium clearly indicates that the widespread mycorrhizal symbiont (or symbionts) known as the E-strain is an ascomycete anamorph. The evidence includes regular septation, the presence of Woronin bodies and injury- and age-induced septal plugs, sensitivity to benomyl, hyphal fusions, mode of hyphal repair, and morphology of the mycorrhizae. Isolates varied in the presence or absence of chlamydospores, pigmentation, growth rates, amount of aerial mycelium, and amount of hyphal ornamentation. It is uncertain whether one or more taxa is currently included in the E-strain concept.

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Des données sur des mycéliums cultivés et des mycéliums provenant du sol montrent clairement que le symbionte mycorhizien commun et connu sous le nom de souche E est un anamorphe ascomycète. Les données pertinentes comprennent un cloisonnement régulier, la présence de corps de Woronin et de "bouchons septaux" provoqués par l'âge ou les blessures, une sensibilité au bénomyl, des fusions d'hyphes, le mode de réparation des hyphes et la morphologie des mycorhizes. Les isolats varient par la présence ou l'absence de chlamydospores, la pigmentation, le taux de croissance, la quantité de mycélium aérien et la quantité d'ornementation des hyphes. On ne sait pas si le concept actuel de souche E comprend un ou plusieurs taxons.

[Traduit par le journal]

Introduction

Mycoorhiza formation by the fungal symbiont referred to be Laiho (1965) as the E-strain is thought to be largely limited to forest nurseries and to be rare in natural forests (Laiho 1965; Mikola 1965), but its abundance in nurseries in Europe and North America suggest otherwise. Evaluations of abundance have been hindered by the absence of a known teleomorph and the lack of definitive criteria that apply to all host–symbiont combinations. Laiho (1965) used the criteria of intracellular infection, a weakly developed mantle, and wide hyphae of the Hartig net to characterize E-strain mycorrhizae of *Pinus* and *Larix*. However, sectioning techniques allow only small portions of mycorrhizal populations to be sampled, and are of limited use in identifying E-strain symbionts if infections are the ectotype. Potentially, the most efficient method of determining the abundance of specific mycorrhizal symbionts is by direct low magnification examination of the mycorrhizae. However, criteria for making such determinations with a high degree of certainty are lacking except for a few very distinctive associations, notably mycorrhizae formed with *Cenococcum geophilum* Fr.

Wilcox *et al.* (1974) discovered large chlamydospores produced by the E-strain fungus and Walker (1979) erected a new genus and species, *Complexipes moniliformis* Walker, to accommodate the E-strain based on similar chlamydospores wet sieved from soil. Walker (1979) tentatively placed this species in the Endogonaceae because it formed chlamydospores similar to those produced by vesicular–arbuscular mycorrhizal species and also because it produced aseptate vegetative mycelium. However, this taxonomic assignment is questionable because chlamydospores occur in all three major divisions of the fungi, and Mikola (1965) and Wilcox (1971) have described E-strain hyphae as septate.

In a study investigating the mycorrhizae of jack pine (*Pinus banksiana* Lamb.) and white spruce (*Picea glauca* (Moench) Voss) planted in mine spoils, it was found that the E-strain was a dominant symbiont. To accurately assess the abundance of the E-strain, a critical examination of the fungus and the mycorrhizae was necessary. This study was aimed at determining what features could reliably be used in identifying E-strain mycorrhizae, the variability of the morphological features of chlamydospores and mycorrhizae, and finally, the taxonomic affinities of the E-strain symbiont(s).

Methods

Mycorrhizae were sampled from two mine spoils which had been transported to Calgary, Alta., and amended with peat, sewage sludge, or fertilizer or left unamended (Parkinson *et al.* 1980). Coal mine spoil from a subalpine spruce–fir site was planted with container-grown white spruce, and extracted oil sand spoil with container-grown jack pine. Quantitative data

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TABLE 1. Cell lengths (micrometres) of hyphae of the E-strain in culture, growing from spruce mycorrhizae and picked from jack pine roots

	Cell position			
Source of hyphae	1	2	3	4
Spruce in peat				
Mean	260*a**	61*a*	57*a*	54*a*
SD†	84	21	15	15
Range	118–393	15–97	34–106	36–91
Spruce in sewage				
Mean	212*b*	78*b*	62*a*	66*b*
SD	54	28	24	19
Range	127–323	29–124	17–124	38–114
Jack pine in peat				
Mean		67‡		
SD		25		
Range		14–132		
MMN agar				
Mean	72	42		
SD	26	13		
Range	30–148	17–70		

*Values in each column followed by the same letter did not differ significantly as determined by the *t*-test ($p = 0.05$).

†SD, standard deviation.

‡Mature cells of unknown positions.

on the mycorrhizae and details of the spoils and amendments will be reported in later publications.

The E-strain was isolated from mycorrhizae of spruce and jack pine on either 2% malt extract agar or modified Melin–Norkrans (MMN) agar (Marx 1969) with 48 ppm sequestrene iron substituted for $FeCl_3$ and glucose for sucrose. To determine the septal condition, cell lengths from four sources were measured. Isolate R-947, isolated from white spruce, was grown on MMN agar and the length of 50 apical and subapical cells on the agar surface measured. Secondly, individual hyphae were picked from mycorrhizal roots of jack pine growing in peat amended oil sands and the distance between 100 septa was measured. The last two sources of hyphae were from E-strain plus white spruce mycorrhizae which were grown in peat or sewage sludge amended spoil. After the mycorrhizae were washed and stored in water at 5°C, E-strain hyphae grew from the mycorrhizae and the lengths of the first four cells of 25 hyphae from each root sample were measured. Hyphal characteristics were observed in water, lactophenol, and Shear's mounting fluid (Chupp 1940) from which most of the ethanol had evaporated.

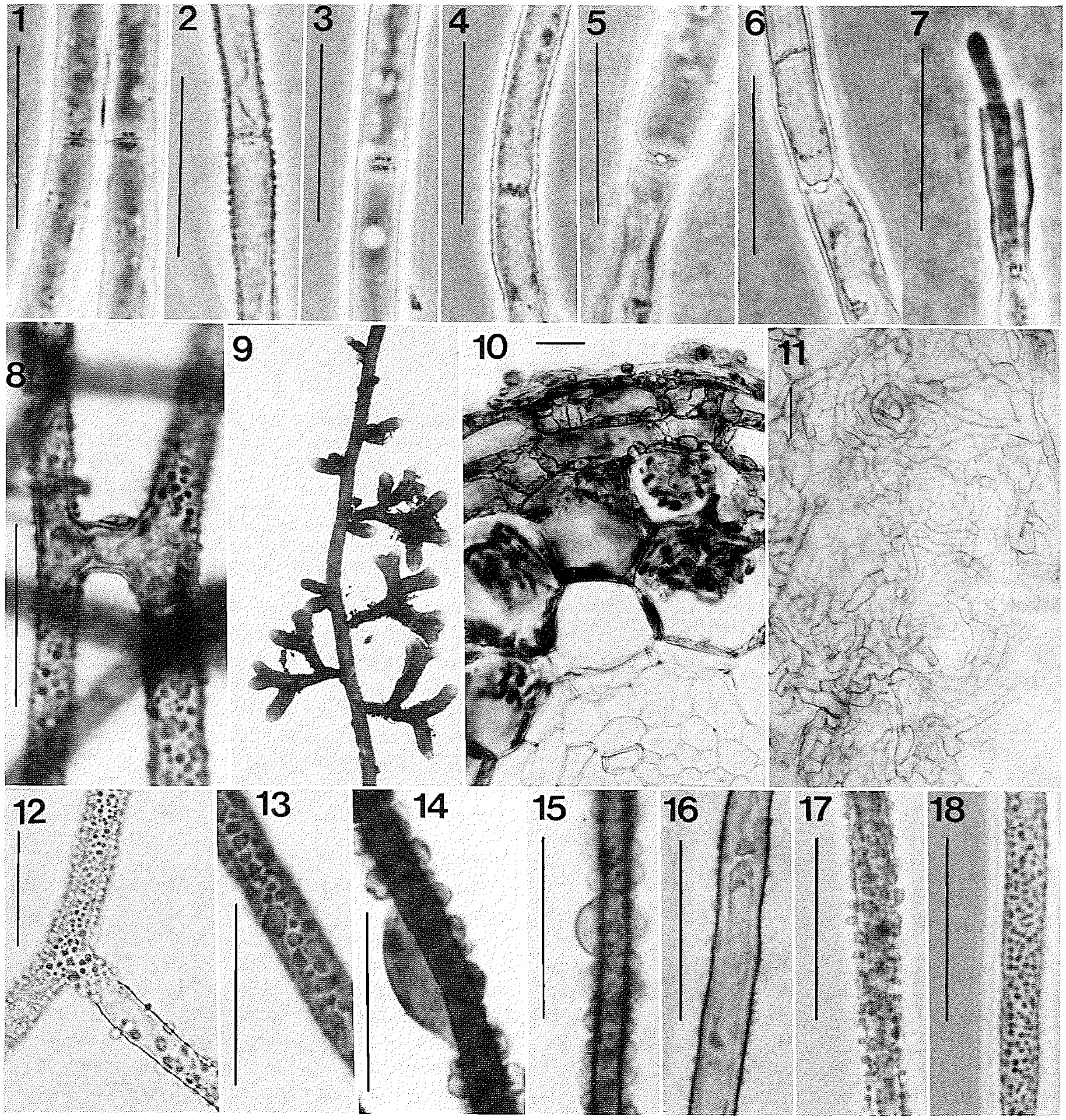
Chlamydospores were extracted from the spoil by wet sieving and collected on 53- and 125-μm sieves. Wet sieving was also used to collect chlamydospores from a single jack pine plus E-strain synthesis jar containing a vermiculite–peat mixture. Dark pigmented chlamydospores were briefly exposed to household bleach so the wall structure could be observed. Chlamydospores were prepared for the scanning electron microscope (SEM) by placing spores on stubs, sputter coating them with gold, and examining them with a Hitachi S450 SEM. Hand-cut sections of mycorrhizae stained with 0.05% trypan blue were examined with a Zeiss photomicroscope and observations recorded on Panatomic-X film. Voucher specimens of chlamydospores and cultures are on deposit at the Biosystematics Research Institute, Ottawa, Canada.

Results

Taxonomic affinity

Septa were present in all hyphae from mycorrhizae and cultures (Table 1). Individual cell lengths of hyphae from roots varied considerably but mean lengths of nonapical cells ranged from 54 to 78 μm. Septal intervals and the distance the growing tip extended prior to septation were shorter in hyphae from culture than hyphae from mycorrhizae. In the subalpine spoil there were significant differences ($p = 0.05$) in cell lengths between the sewage and peat amendments.

Isolate R-947 was grown on several media and aerial, surface, and submerged hyphae of various ages were examined for septal characteristics using phase-contrast microscopy. Septa were most easily seen in the terminal two or three cells of surface hyphae. Water and Shear's mounting fluid were superior to lactophenol for microscopy as cell contents coalesced in lactophenol. Two to six globose microbodies, 0.2–0.4 μm in diameter, were consistently associated with young septa (Fig. 1) and were identical to Woronin bodies (Buller 1933; Reichle and Alexander 1965). Woronin bodies could rarely be seen in ornamented hyphae using phase contrast (Fig. 2)



Figs. 1, 2. Woronin bodies of R-947 on MMN. Figs. 3, 4. E-strain hyphae from spruce mycorrhizae with Woronin bodies. Fig. 5. Injury-induced septal plug of R-947. Fig. 6. Age-induced septal plug of R-947. Fig. 7. Renewed growth in injured hyphal cell of R-947. Fig. 8. Hyphal fusion of R-947; stained with cresyl blue. Fig. 9. E-strain jack pine mycorrhizae from peat amended oil sand spoil. Fig. 10. Cross section of E-strain jack pine mycorrhiza; stained with trypan blue. Fig. 11. Surface of E-strain jack pine mycorrhiza. Fig. 12. Ornamented E-strain hypha, unstained. Figs. 13, 14, 15. Ornamented E-strain hyphae stained with methylene blue. Fig. 16. Ornamented E-strain hypha, unstained. Fig. 17. Ornamented E-strain hypha from spruce plot, stained with methylene blue. Fig. 18. Ornamented E-strain hypha from spruce plot, stained with trypan blue. Figs. 1–18. Scale lines equal 20 μm.

but they stained with hot trypan blue and could then be seen with bright-field optics. Woronin bodies were also observed in young and ornamented hyphae associated with spruce mycorrhizae (Figs. 3, 4).

Septal pores cannot be seen using light microscopy but their presence can be inferred by observing other phenomena. The strongest evidence for a pore was the presence of injury-induced and age-induced septal plugs. When hyphae from the edge of a colony were cut, the septa between injured and sound cells always had thickened central areas (Fig. 5). Most septa from old portions of colonies lacked Woronin bodies and had conspicuous thickenings at the centre, which were interpreted as age-induced septal plugs (Fig. 6). Hyphae from roots also had central thickenings of the septa which appeared to be age-induced plugs.

Other indicators of taxonomic affinity included mode of hyphal repair, hyphal fusions, and sensitivity to the fungicide benomyl. After injury, hyphae resumed growth by producing an eccentrically located swelling on the last intact septum and the subsequent growth of a small hypha within the old injured hypha (Fig. 7). Hyphal fusions were rare but occasionally occurred in aerial hyphae of E-strain cultures (Fig. 8). Six isolates of the E-strain, two Basidiomycetes (*Suillus tomentosus* Sing., Snell & Dick and *Lactarius paradoxus* Beardslee & Burlingham), an ascomycete (*Sphaerosporella brunnea* (Alb. & Schw. ex Fr.) Svrcek & Kubicka), and an ascomycete anamorph (*Cenococcum geophilum* Fr.) were tested for sensitivity to benomyl. The Basidiomycetes were the only fungi that grew on media containing benomyl (Table 2).

TABLE 2. The effects of benomyl on the growth of six E-strain isolates, an ascomycete, an ascomycete anamorph, and two basidiomycetes*

Isolates	Radial growth after 20 days (mm) at the following benomyl concentrations (mg/L): 0	1	5
R-947	19.0	0	0
R-977	5.3	0	0
R-1302	6.3	0	0
R-1312	17.0	0	0
R-1706	11.0	0	0
R-1719	6.6	0	0
Sphaerosporella brunnea	>70	0	0
Cenococcum geophilum	7.0	0	0
Lactarius paradoxus	8.0	8.0	8.3
Suillus tomentosus	13.3	11.6	13.0

*The basal medium was modified MMN agar with 0, 1, and 5 mg/L of active ingredients of benomyl. Three replicate plates were inoculated with agar plugs taken from the margin of actively growing colonies.

Criteria for identification

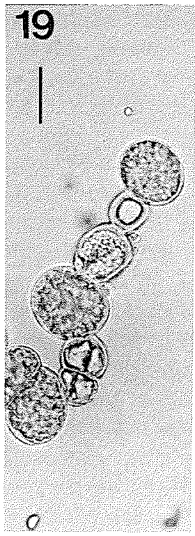
Mycorrhizae

Jack pine plus E-strain mycorrhizae were monopodial, dichotomous, or bifurcately branched two or three times (Fig. 9). The colour was pale to dark brown, cigar brown, or darker with a glabrous surface and sparse extramatrical brownish hyphae. A mantle was lacking or very thin, the Hartig net continuous throughout the cortex and nearly all cortical cells were filled with smooth, septate hyphae (Fig. 10). Thus the only anatomically distinctive feature of E-strain plus jack pine mycorrhizae was the intracellular hyphae. The mycorrhizae formed with spruce and the E-strain were of the ecto-type and monopodial. The most useful feature in recognizing the E-strain regardless of host was the large size of the hyphal elements on the mycorrhizal surface. The hyphae were 4–8 μm in diameter and readily observed in whole mounts (Fig. 11). These hyphae are larger than those of most other mycorrhizal fungi and an ascomycetous affinity could be confirmed by observing Woronin bodies in young hyphae near the root meristem using a 40× bright-field objective. The type of ornamentation of the extramatrical hyphae, and the presence of E-strain chlamydospores (though rarely seen attached to mycorrhizae) were less useful criteria.

Hyphae

The most common appearance of the surface of the aerial hyphae of R-947 on MMN agar was verrucose, i.e., densely ornamented with rounded processes about 0.5–1 μm in diameter (Figs. 12, 13). The ornaments were not simple thickenings of the wall or external incrustations but rather appeared to be produced by the separation of a very thin outer wall layer from a structural inner wall. The blisterlike nature of the ornaments was most apparent where large areas of the outer wall had separated from the inner wall (Figs. 14, 15, 16). Methylene blue strongly stained the blister contents of some hyphae but the contents of other ornaments remained hyaline. Ornaments were present on aerial hyphae when R-947 was grown on potato dextrose agar (PDA), Leonian's pycnidial agar (Leonian

FIG. 19. Chains of chlamydospores produced in agar. FIG. 20. Chains of chlamydospores on the agar surface. FIG. 21. Young chlamydospore formed on MMN. FIG. 22. Same chlamydospore as in Fig. 21 after staining with methylene blue. FIG. 23. Young, thick-walled chlamydospore formed on MMN. FIGS. 24, 25, 26. Chlamydospores formed on MMN. FIGS. 27, 28, 29, 30, 31. Chlamydospores produced in jack pine synthesis jar. Figs. 19–31. All scale lines equal 20 μm except Fig. 20 in which it equals 100 μm.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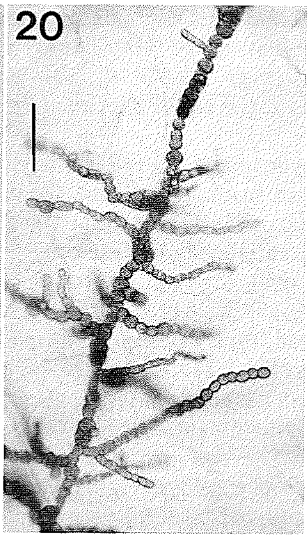
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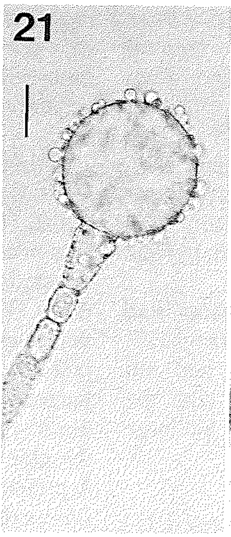
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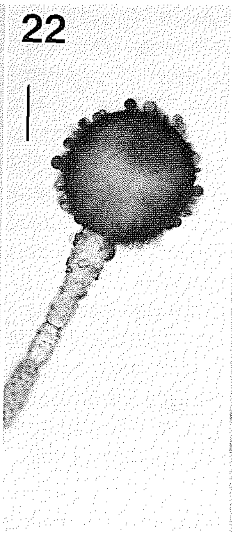
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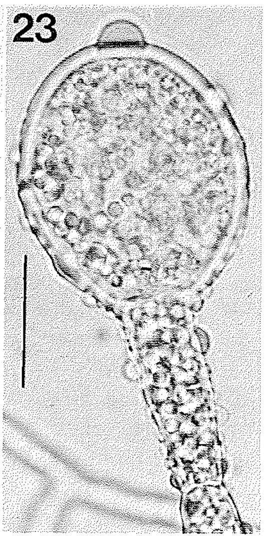
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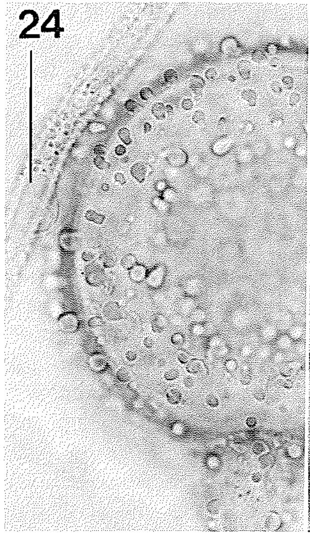
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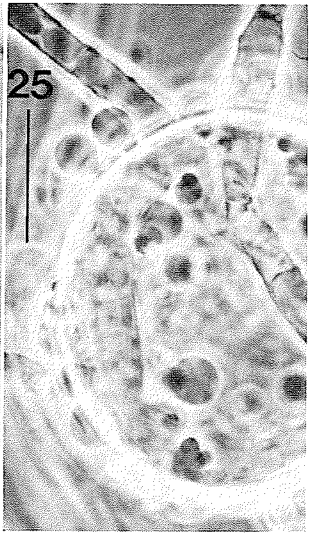
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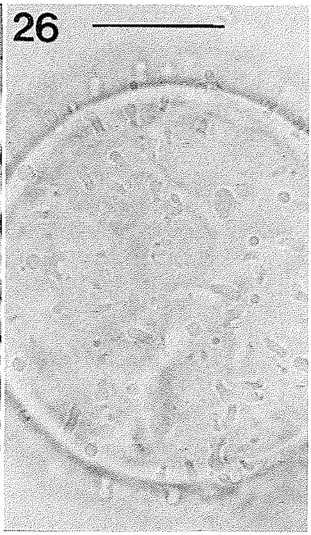
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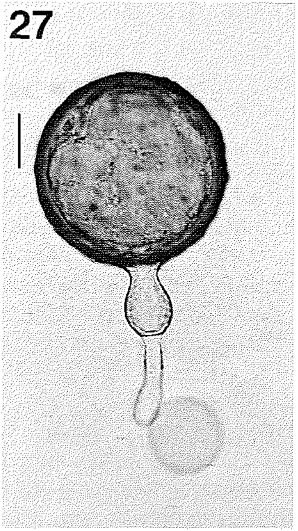
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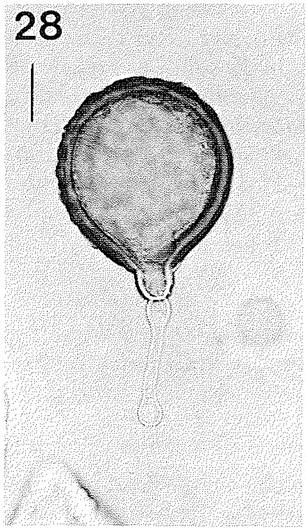
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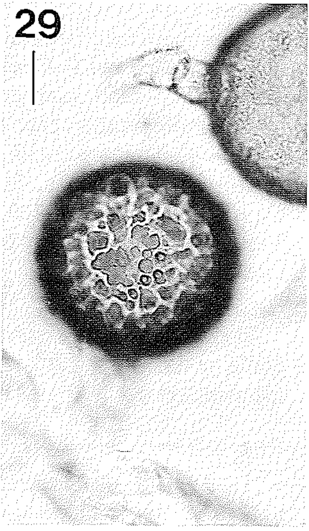
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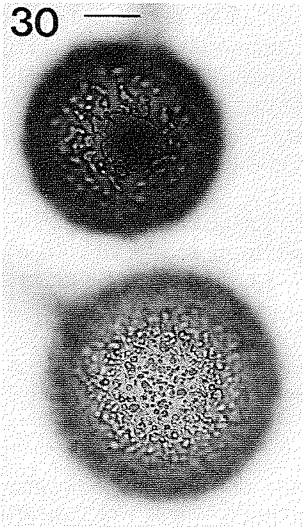
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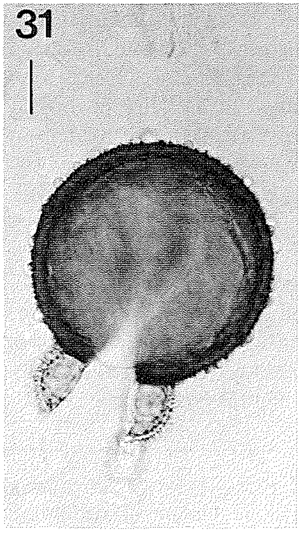
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1924), and GYMA (Stevens 1974) but the hyphae were smooth on V-8 agar. Ornaments were not formed on mantle or intracellular hyphae of mycorrhizae or on hyphae submerged in agar. A test of the effect of submergence on ornamentation was conducted by growing R-947 on MMN agar and either leaving the inoculum exposed or covering it with sterile water or sterile water and mineral oil. Ornamentation was completely suppressed in submerged hyphae whereas aerial hyphae developed blister-type ornaments.

Hyphae around white spruce seedling roots had ornaments similar to those produced in culture (Figs. 17, 18). They were 3–5 μm in diameter, smooth, and thin walled when young, becoming ornamented with the structural wall up to 1 μm thick when mature. No large blisters were observed on hyphae from soil.

Chlamydospores

Chlamydospores from three sources were studied: (1) MMN agar plates, (2) a synthesis jar, and (3) mine spoil planted with white spruce. Two types of chlamydospores were formed on MMN agar plates; a distinctive terminal type borne in aerial positions and chains of intercalary spores in the agar. The second type was smooth with thickened walls (Fig. 19) and identical to those illustrated by Wilcox *et al.* (1974, Figs. 8, 9). Submerged hyphae and hyphae on the agar surface often consisted entirely of subglobose cells with thin, hyaline walls or thick, pale brown walls (Fig. 20). These spores have less diagnostic value than the terminal, ornamented chlamydospores and were not considered further.

Sixteen of 46 isolates tested produced aerial chlamydospores on MMN agar. The chlamydospores were borne on erect chains of subtending hyphae up to 400 μm tall that originated from the agar surface. The chlamydospores were globose, 50–80 μm in diameter, hyaline to pale brown, and verrucose (Figs. 21, 22). In all cases the ornaments appeared to be formed by a separation of a membranous outer wall layer (Fig. 23). The ornaments were most commonly convex, occasionally clavate (Fig. 24) or pyriform (Fig. 25), and rarely digitate (Fig. 26) or capitulate. The ornament contents were hyaline to ochraceous and often stained deeply in methylene blue (Fig. 22).

Chlamydospores of R-947 formed in the vermiculite–peat synthesis jar varied from nearly smooth to richly ornamented (Figs. 27–33, 48, 49). The darker pigmentation of the wall indicated that they matured further than those formed on plates. Spores with a nearly smooth wall (Figs. 27, 28) were rare as were those with angular ornaments (Figs. 29, 30). A few spores had ridgelike ornaments but most spores had convex blisterlike ornaments (Fig. 31) or most commonly digitate ornaments (Figs. 32, 33, 48, 49). Many of the digitate ornaments had a small depression at the tip (Figs. 33, 49).

Chlamydospores sieved from soil were globose, 80–120 μm in diameter, and sienna, rusty tawny, or umber (Figs. 34–39, 50–53). The form of the ornaments was variable but a majority of the spores were covered with digitate projections 4–6(10) μm long (Figs. 34, 35, 36, 50, 52, 53). These ornaments were unbranched, thin walled, occasionally constricted several times (Figs. 34, 52) with straw, ochraceous, or rust contents. The ornaments occasionally stained blue–black with methylene blue but were often nonreactive to the stain. The tips of many digitate ornaments were depressed and cupulate (Figs. 36, 52, 53) as was the case for spores sieved from the vermiculite–peat medium. A minority of the soil-borne chlamydospores had convex ornaments similar to those produced in culture (Figs. 38, 39) or a mixture of digitate processes and blisters 2–6 μm broad.

Details of chlamydospore walls could not be seen with heavily pigmented and densely ornamented spores until treated with bleach. All spores had a thickened inner wall (Fig. 40) (the outer wall considered here to be the membranous layer). In optical cross section the inner wall was usually homogeneous but occasional spores had distinctly laminated walls. The inner (structural) wall of a few chlamydospores consisted of two similar layers (Figs. 41, 42, 43) or rarely of three to five laminated layers (Fig. 44). The chlamydospores were brittle and in a few cases fractured walls appeared micalike (Fig. 45). With the exception of one spore, no evidence of a pore or discontinuity of the wall was observed where the spore was attached to the subtending hyphae.

The subtending hyphae consisted of as many as 20 cells in culture and 1–6 cells in the soil. The ornamentation was lenticular, convex, or hemispherical regardless of the ornamentation type occurring on the terminal cell (Fig. 46). The subapical cell was cupulate, urnulate, doliform, or cylindric, 20–28 μm in diameter with a wall thickness of 1–1.5 μm (excluding ornaments). Very rarely the spores were borne in intercalary positions (Fig. 47).

Cultures

The E-strain was cultured from mycorrhizae and could be recognized at a very early stage as it grew from mycorrhizal root tips. The hyphae were stiff, infre-

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FIGS. 32, 33. Chlamydospores produced in jack pine synthesis jar. FIGS. 34, 35, 36. Chlamydospores sieved from spruce soil. FIG. 37. Ornament on soil-borne chlamydospore with depressed tip. FIGS. 38, 39. Chlamydospores sieved from spruce soil. FIG. 40. Wall of bleached chlamydospore. FIGS. 41, 42. Two-layered chlamydospore wall of R-947 in culture. FIG. 43. Three-layered wall of bleached chlamydospore from spruce soil. Figs. 32–43. All scale lines equal 20 μm.

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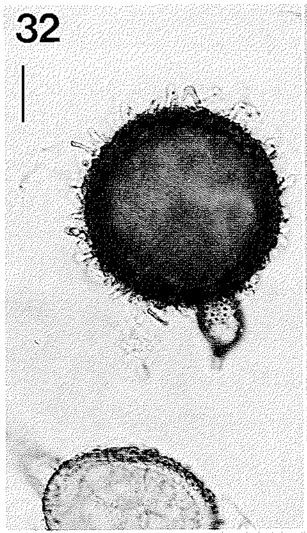

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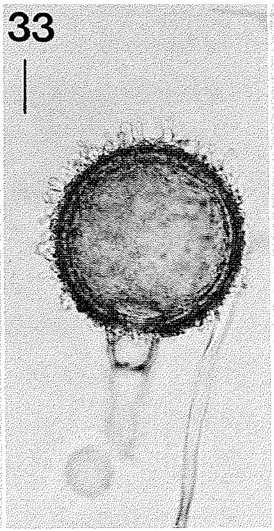

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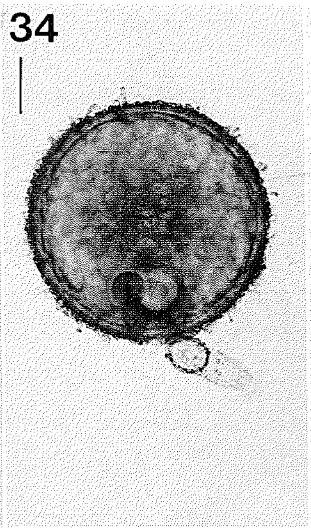

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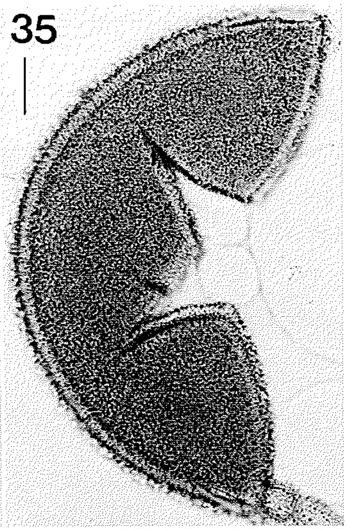

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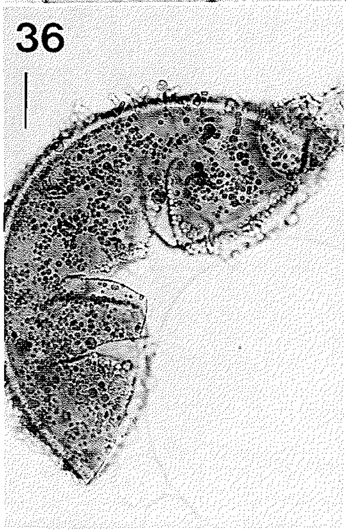

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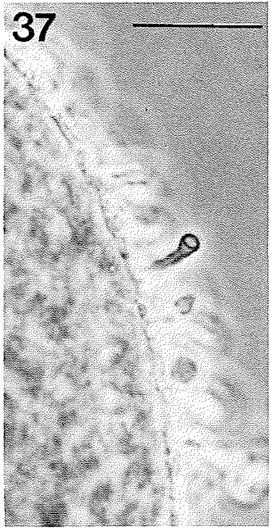

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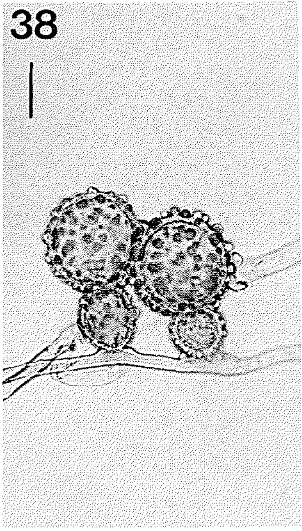

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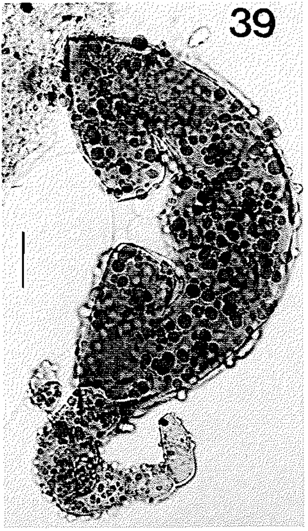

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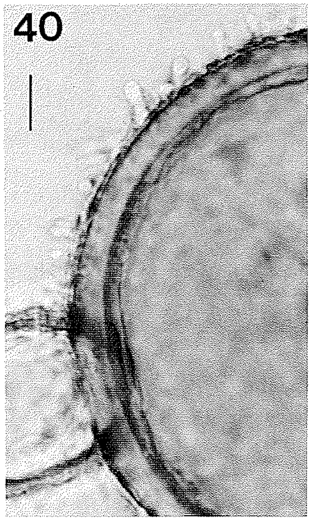

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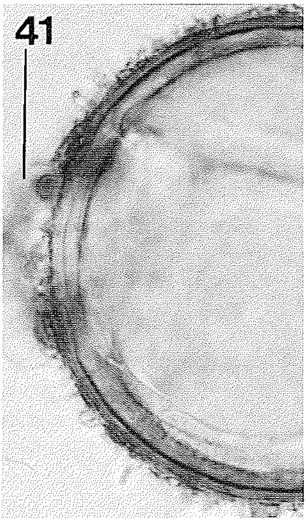

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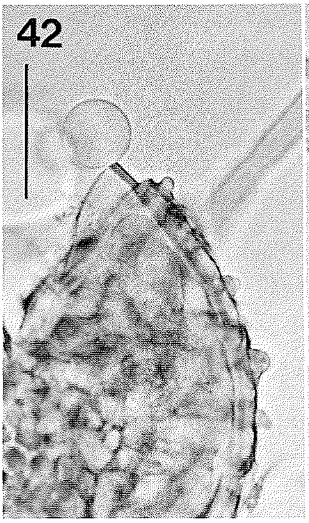

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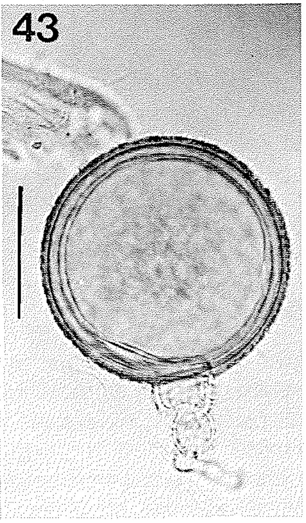
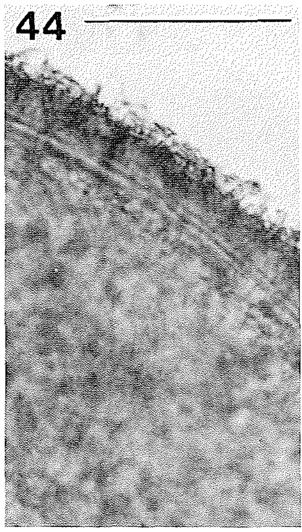
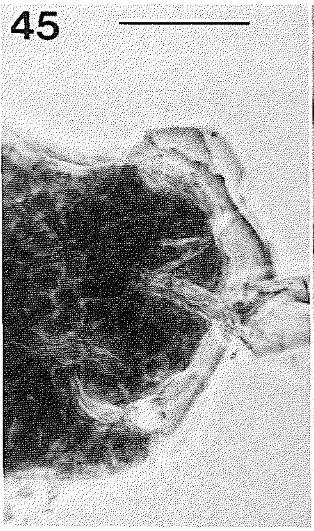
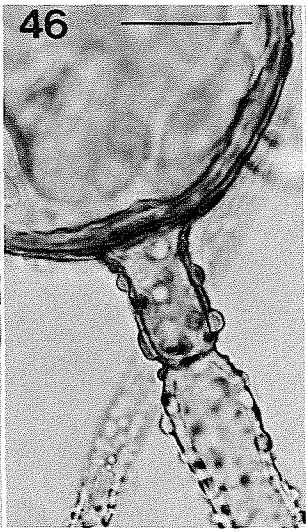
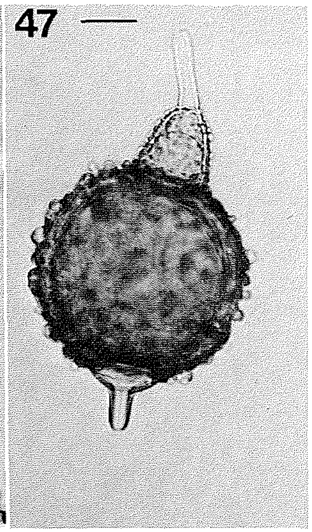
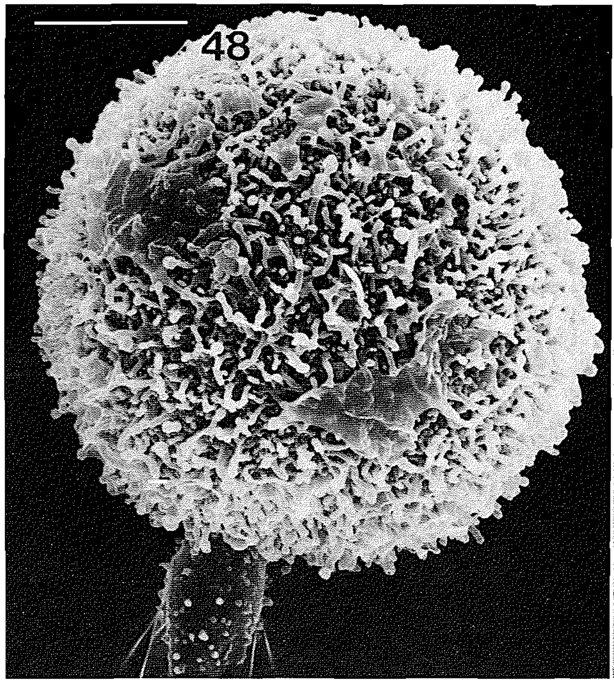












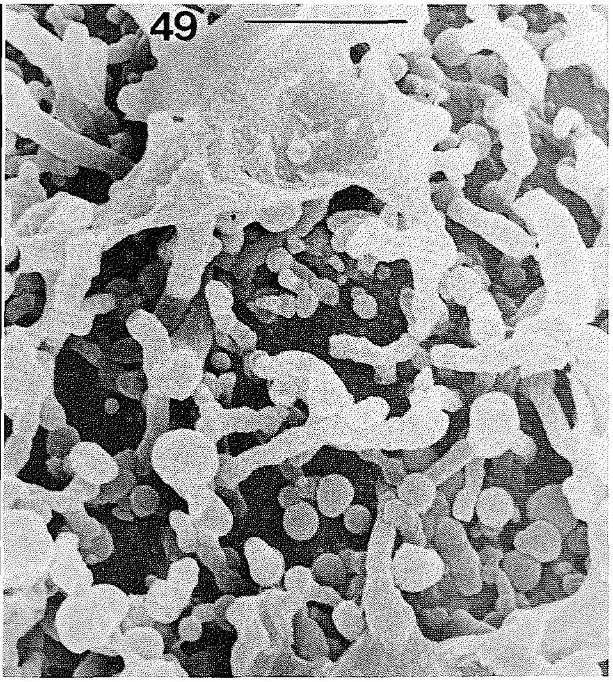
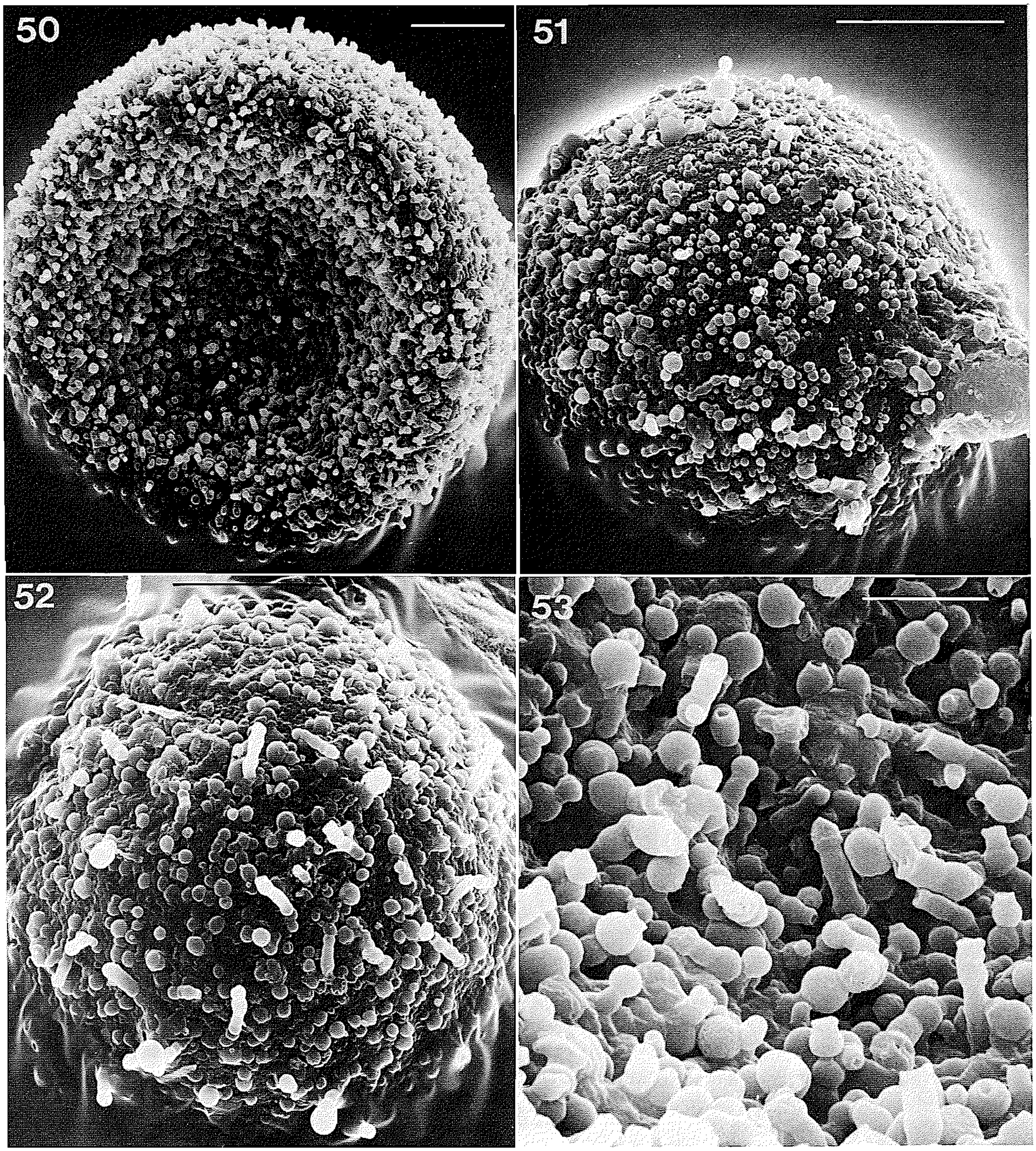


FIG. 44. Multilayered wall of soil-borne chlamydospore. FIG. 45. Fractured wall of soil-borne chlamydospore with five layers. FIG. 46. Subtending hypha of cultured chlamydospore. FIG. 47. Chlamydospore produced in synthesis jar in an intercalary position. FIG. 48. SEM micrograph of chlamydospore from synthesis jar. FIG. 49. SEM micrograph of chlamydospore ornaments. Note depressions on tips of digitate processes. Figs. 44–49. All scale lines equal 20 μm except Fig. 49 in which it equals 5 μm.

quently branched, white to pale brown with a frosted appearance. As colonies aged, the diversity of colony characteristics became pronounced. Colony diameters ranged from 10 to 50 mm after 25 days growth. Chlamydospores were produced by certain isolates on MMN agar but other isolates failed to produce either aerial or submerged chlamydospores. Cultures of chlamydospore-producing isolates were pale brownish yellow to very faint brown with arachnoid margins and floccose or with sparse aerial mycelium. Submerged chains of chlamydospores were usually present. Non-chlamydospore producers had even colony margins, rapid to slow growth rates, and were either floccose and hyaline, hyaline with sparse aerial mycelium, or



FIGS. 50–53. SEM micrographs of soil-borne chlamydospores. FIG. 50. Small digitate type of ornamentation. FIG. 51. Convex ornaments. FIG. 52. Mixture of convex and digitate ornaments. Note constrictions and apical depressions of digitate ornaments. FIG. 53. Enlargement of Fig. 50 showing details of digitate ornaments. Figs. 50–53. All scale lines equal 20 μm except Fig. 53 in which it equals 5 μm.

dark brown, slow growing, and with abundant stiff ornamented aerial mycelium. All colony types included here as E-strains had some stiff, ornamented hyphae.

Discussion

Wilcox *et al.* (1974) were the first to note the similarity of the chlamydospores of the E-strain to those of endogonaceous fungi and Walker (1979) tentatively placed it in the Endogonaceae as *Complexipes moniliformis*. Observations reported here on septal characteristics strongly suggest an alternate taxonomic choice, that of an ascomycete anamorph. Moore (1978) proposed that the lower and higher fungi could be separated on the basis of the presence or absence of regular septation and that the higher fungi could be further divided depending on whether the septa were simple with associated Woronin bodies or of the dolipore type, thus following classical taxonomic lines with nonreproductive criteria. Septa were regularly spaced in the fungi observed in this study and the presence of Woronin bodies and hyphal plugs are good evidence for an ascomycetous affinity. This is further supported by the secondary characters of hyphal fusion, mode of hyphal repair, and sensitivity to benomyl. Burnett (1976) stated that hyphal fusions are absent in phycomycetes but Warcup (1975) reported frequent hyphal fusions in a species of *Endogone*. The growth of hyphae of R-947 after injury conformed to that reported by Buller (1933) for an ascomycete and was unlike the "bridging" growth illustrated by Gerdemann (1955) and Sward *et al.* (1978) for endomycorrhizal fungi. The toxicity of benomyl to the E-strain strongly indicates an ascomycetous affinity (Hall 1979).

The presence of septa in hyphae of the E-strain agrees with observations by Levisohn (1954), Laiho (1965), Mikola (1965), Wilcox (1971), Wilcox *et al.* (1974), and Thomas and Jackson (1979). However, Hall (1977) and Walker (1979) reported that hyphae associated with E-strain type chlamydospores were aseptate and Fig. 1E in Walker (1979) illustrates a length of smooth hyphae about 400 μm long lacking septa. As considerable lengths of hyphae were measured in this study, and the longest subapical cell was 132 μm long, it is unlikely that a 400-μm length of hyphae of the E-strain would be aseptate. Another possibility is that two quite different fungi produce similar chlamydospores. The very dark pigmentation of the spores makes critical comparisons of surface features and wall characteristics from published photographs impossible. Mosse and Bowen (1968) and Hall (1977) described spores similar to E-strain chlamydospores and stated there was an open pore at the base of the chlamydospore, a condition seen only once in this study where hundreds of spores were studied critically. Additional observations are required on chlamydospores resembling those described in this study to determine if more than one taxon produces similar spores.

Differences in cultures indicate more than one taxon may be included in the E-strain concept. Ectendomycorrhizae were formed with jack pine with both chlamydospore-forming isolates and nonchlamydospore formers (e.g., slow growing, dark forms) but additional field sampling is required to determine if these represent different taxa. This cannot be resolved with certainty until the teleomorph(s) is discovered. Owing to the apparent anomolies of hyphal septation, presence or absence of a pore between chlamydospores and subtending hyphae, and cultural variations, the orignal trivial name, E-strain, is retained here regardless of taxonomic composition. Application of the name *Complexipes moniliformis* can only be made to chlamydospore-producing isolates.

Recognizing the anatomy of the hyphal ornaments as blisterlike may aid in the identification of cultures as well as mycelium in soil. Similar wall ornaments have been illustrated for a basidiomycete with the suggestion that the ornaments were a repository for waste products (Chilvers 1968; Sevior *et al.* 1978). In this study blister formation did not occur on submerged, mantle, or intracellular hyphae, possibly indicating that roots and water may serve as sinks for diffusible waste products. Transmission electron microscopy is required to determine if E-strain ornaments are similar to the pellicle-bound ornaments described by Campbell (1976) and Evans and Stempen (1980).

The form of the chlamydospore ornaments was found to be quite variable with material from one soil source. The ornaments appeared to result from the deformation of a thin, plastic outer wall layer which produced a variety of forms. Wall structure was also variable and does not appear to be useful in identifying E-strain chlamydospores. The thick wall often produced optical haloes which were difficult to distinguish from true wall discontinuities.

Jack pine mycorrhizae had large amounts of intracellular hyphae as has been observed with other species of pine (e.g., Laiho 1965). These hyphae were smooth, septate, and equal in diameter as illustrated by Mikola (1965, Fig. 3) with no suggestions of arbuscular form or hyphal digestion. Intracellular hyphae in red pine appear unusual in that they were constricted and beaded (Wilcox *et al.* 1974, Fig. 34). Such variations should not be unexpected as submerged hyphae in this study were often beaded while aerial hyphae of the same culture were cylindrical.

The absence or very reduced development of a mantle appears to be characteristic for pines in the field but this feature is subject to considerable variation. In pine synthesis flasks, the mantle can be very thick (Wilcox *et* *al.* 1974) and in nursery-grown spruce, moderately well developed (Thomas and Jackson 1979). The characteristics of the extramatrical hyphae, size of the surface hyphae, and presence of Woronin bodies should allow more precise identification of mycorrhizae collected in the field. However, it is likely that closely related ascomycetes may form similar mycorrhizae and the symbiont should be cultured to confirm the identity of the E-strain.

Although the teleomorph of the E-strain is unknown, examination of probable ascomycete symbionts indicates what taxa should be searched to compare with the E-strain. It is felt that species of *Geopora, Trichophaea, Sphaerosporella*, and *Humaria* are likely candidates for an E-strain teleomorph(s). Attempts to culture *Geopora clausa* (Tul. & Tul.) Burdsall from pieces of the ascocarp failed but a few hyphae did grow from the inoculum. These hyphae were identical to E-strain hyphae in terms of ornamentation and size. Herbarium specimens of *Geopora cooperi* Harkness and *G. pellita* (Cooke & Peck) Schumacher had large diameter, ornamented hyphae. It is anticipated that mycorrhizae formed by *Geopora* species would be similar to those formed by the E-strain. Although species of the other three genera mentioned above are not recognized as mycorrhizal symbionts, one species, *Sphaerosporella brunnea*, has been confirmed as a symbiont of jack pine and is currently under study (unpublished data). It would be desirable to culture species in these genera in hopes of discovering the E-strain teleomorph and possibly additional symbionts in a group of fungi that has been largely neglected in terms of symbiotic relationships.

Detailed examination of the E-strain in this study indicates that additional criteria are available to characterize and identify mycorrhizal symbionts. Efforts should be made to determine ascomycete or basidiomycete affinities of nonclamp-bearing species so a taxonomic distribution of symbionts can be obtained. The work of Wilcox and co-workers (Wilcox *et al.* 1974) has implicated previously ignored fungal taxa as symbionts but information on hypogeous ascomycetes and other ascomycetes, as shown by mycorrhizal frequency rather than sporocarp occurrence, is acutely needed to clarify their importance in forest ecosystems.

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